

## EMBRYONIC DEVELOPMENT IN POECILIID FISHES

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In *Heterandria formosa*, a viviparous cyprinodont fish of the family Poeciliidae, nearly all of the nourishment for embryonic development is obtained from the mother after fertilization through a pseudoplacental association (Scrimshaw, 1944). Similar although less complicated associations between mother and embryo exist in other species of this family (Turner, 1937, 1940). These species are for the most part considered to be ovoviviparous. The distinction is based on the difference between live bearing forms which retain an egg with a full supply of nourishment for development and those whose embryos receive nourishment from the mother. The latter are considered truly viviparous.

Turner (1937) referred to all species of Poeciliidae as ovoviviparous. He obviously used this term in a general sense for he suggested in discussing the data of Bailey (1933) that *Xiphophorus helleri* could receive nutriment from the parent. Turner also pointed out (1937) that the small *Heterandria* egg cannot contain enough nourishment to account for the size of the larvae and suggested that the follicle cells surrounding the embryo furnish food materials.

The weight values reported by Bailey for various embryonic stages in *Xiphophorus* show no decrease in the weight of the total yolk-embryo system. Since energy is used for maintenance metabolism, the total weight of this system decreases in forms depending entirely on yolk. Gray (1928) reported a decrease of 37 per cent for the oviparous trout, *Salmo fario*, and Hsiao (1941) found a decrease of 34 per cent in the truly ovoviviparous perch, *Sebastes marinus*. Accordingly, although he does not suggest this, Bailey's data show that some nourishment must be obtained from the mother by the developing embryo of *Xiphophorus*.

It is true that most poeciliid fishes are more dependent on the yolk laid down before fertilization than upon maternally supplied nourishment after that time. However, the evidence presented below shows that the members of this family do utilize nourishment outside of that contained in the yolk and hence are not ovoviviparous in the strict sense of the term. Embryos of such species as *Heterandria formosa* and *Aulophallus elongatus* are truly viviparous and as dependent on the mother for nourishment as are those of a placental mammal.

Gray (1926, 1928) reported the relationships between nourishment and growth rate in the oviparous trout, *Salmo fario*. The problems of oviparity and ovoviviparity are similar in that in each the embryo has its own supply of nourishment and receives food and water from its environment. When these conditions are compared with true viviparity, striking differences are noted (Scrimshaw, 1944). The present study was undertaken to find and describe intermediate stages between

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viviparity and ovoviviparity in other members of the family. Such stages would aid in the understanding of the complex problems of viviparity in this specialized group. The weight changes in the egg and embryos of twenty additional species of Poeciliidae have been determined and a condition intermediate between ovoviviparity and true viviparity characterized. There appeared a remarkable tendency for the young of all the species examined to receive from the mother only as much in terms of dry weight as they required for the maintenance of tissues already formed.

#### MATERIAL

Stocks of the following species were maintained in the laboratory and the gravid females were available as desired. These were dissected and the living embryos weighed.

<i>Gambusia affinis holbrookii</i>	<i>Quintana atrizona</i>
<i>Lebistes reticulatus</i>	<i>Xiphophorus helleri</i>
<i>Poeciliastes pleurospilus</i>	

The following species were collected in the field and the embryos weighed at the Institute for Research in Tropical America, Barro Colorado Island, Canal Zone (June–August, 1940).<sup>2</sup>

<i>Allogambusia tridentiger</i>	<i>Brachyrhaphis episcopi</i>
<i>Aulophallus clongatus</i>	<i>Darienichthes dariensis</i>
<i>Brachyrhaphis cascajalensis</i>	<i>Mollicnesia sphenops</i>

In addition the writer is greatly indebted to Dr. Carl Hubbs of the Museum of Zoology, University of Michigan, for preserved material of the following species:

<i>Allophallus kidderi</i>	<i>Poecilia vivipara</i>
<i>Beloncosox belizanus</i>	<i>Poeciliopsis infans</i>
<i>Brachyrhaphis terrabensis</i>	<i>Poeciliopsis occidentalis</i>
<i>Dextripenis evides</i>	<i>Poeciliastes pleurospilus</i>
<i>Gambusia nobilis nobilis</i>	<i>Pseudoxiphophorus bimaculatus</i>
<i>Mollicnesia velifera</i>	<i>tacniatus</i>

Through the kindness of Dr. William Schroeder, additional specimens of *Gambusia affinis holbrookii* were obtained from the Museum of Comparative Zoology of Harvard University.

The gravid females of all of the above species were dissected and the contents of the ovary noted. In all of these forms the embryos are retained within the follicles of a large single median ovary which was removed intact before examination.<sup>3</sup> The embryos could be dissected out with the ovisac intact and the ap-

<sup>2</sup> This work was made possible by a grant from the Penrose Fund of the American Philosophical Society and the assistance of Dr. Thomas Barbour of the Museum of Comparative Zoology, Harvard University. The author was greatly assisted in the field work and weight measurements by Mr. Thomas C. Saunders of Harvard University. The cooperation of Mr. James Zetek, custodian of the island and laboratory; Mr. Graham Fairchild of the Gorgas Memorial Laboratory, Panama; and Mr. John Wood of the Canal Zone Police considerably aided this work.

<sup>3</sup> Dissection and weight data for all of these species are contained in the appendix of the author's doctorate thesis *Embryonic Growth in Poeciliid Fishes* deposited in the Harvard University Library.

proximate diameter measured with an ocular micrometer. The weight determinations were made as described for *Heterandria* (Scrimshaw, 1944), the embryos being dried on small cover slips over calcium chloride and weighed on a Sartorius balance. Any error in the actual dry weight recorded was insignificant in comparison with the variability of the material. Wet weights are not discussed because of their variability in preserved material and the difficulties in determining excess fluid. The taxonomic scheme followed throughout is that proposed by Hubbs (1924, 1926).

### EXPERIMENTAL

Dry weight determinations of the eggs and embryos in the intact ovisac were made at various stages between fertilization and parturition. In each case as many stages as could be obtained were studied and ranked according to age as described below for purposes of graphical and statistical representation.

It was soon observed that these weights were within the same general range regardless of the stage of the embryo. When least square lines were calculated through these points, a straight line parallel to the abscissa served to describe the data. Figures 1 and 2 show representative development lines and the scatter of weight values for four of these species.

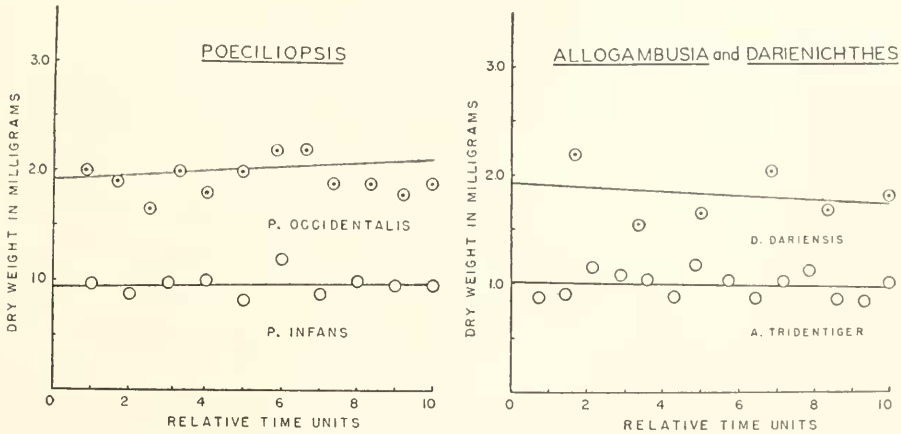


FIGURE 1. The dry weights of the embryos in the intact ovisac are arranged on a relative time axis in order of stage of development. For graphical and statistical purposes it has been necessary to assume that the time between stages is equal in all cases. Fertilization is represented at the beginning of the time scale and parturition at the end. The lines are calculated through these points by the method of least squares.

FIGURE 2. Two closely related species are portrayed in the same manner as those in Figure 1.

On the basis of these straight lines, the mean weight of the embryos of this group of poeciliid species can be compared (Figs. 3 and 4). The range is so great that all of the species could not be represented in one figure, but several forms are repeated in Figure 4 to facilitate comparisons. In addition to the species described

in this paper, *Heterandria formosa* and *Aulophallus elongatus*<sup>4</sup> are included as examples of viviparous poeciliid fish securing nearly all of their nourishment from the mother. The straight line for *Aulophallus* is purely diagrammatic, but its use

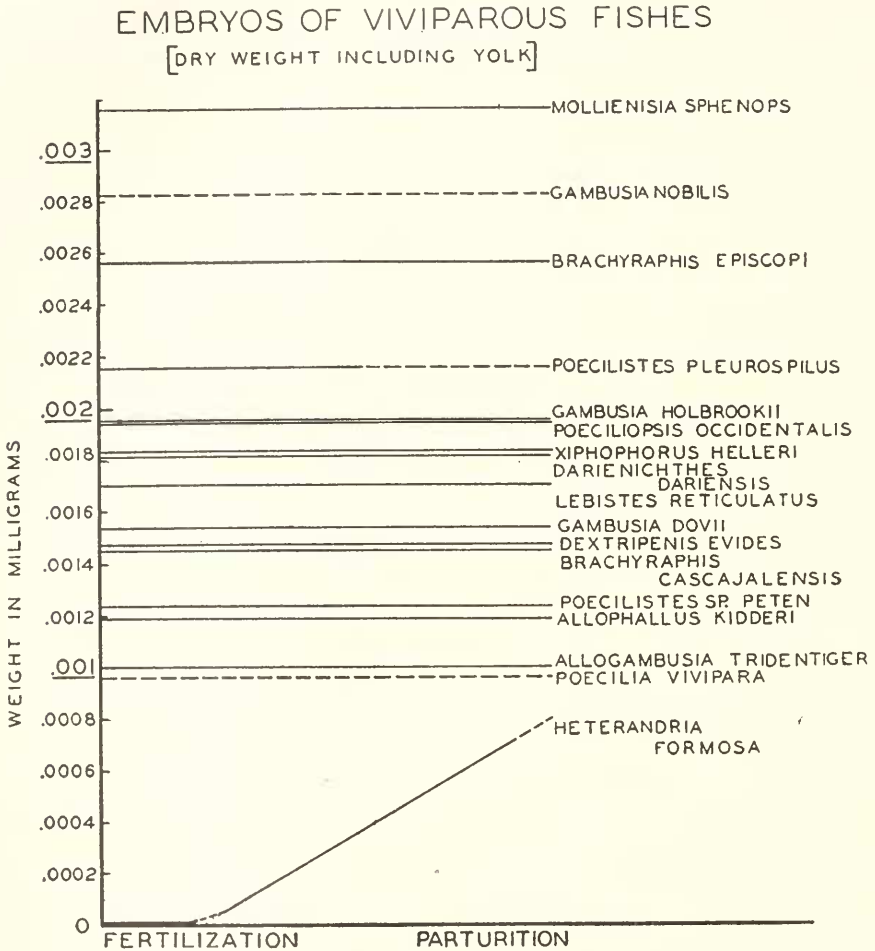


FIGURE 3. For purposes of comparison the dry weight curve of the embryo during development is drawn as a horizontal line. The average weight of all of the embryos of a given species is taken as the level at which the line is drawn. The degree to which these diagrammatic lines correspond to lines calculated through the data is discussed below. An approximation of the standard deviation observed from the above lines for each species is given by  $SE_y$  in Table I. *Heterandria formosa* is included as an example of a truly viviparous fish. The other species have previously been considered to be ovoviviparous. *Gambusia holbrookii* should be listed as *Gambusia affinis holbrookii*. *Poecilistes* sp. Peten has been identified by Hubbs as *Poecilistes pleurospilus* from Guatemala.

<sup>4</sup> Measurements on this species were also made in Panama during the summer of 1940. The weight of the fertilized egg and of the larva at parturition are indicated in Figure 1. A more complete discussion of this species is being prepared.

for *Heterandria* is probably legitimate (Scrimshaw, 1944). The broken lines indicate incomplete data. The known oviparous fishes have egg and larva weight greater than those of any of the fishes shown in Figure 1.

In Figures 1-6 no correction has been made for the effect of the fixative on the dry weight of the embryo. As can be seen from Figure 5, no single correction can

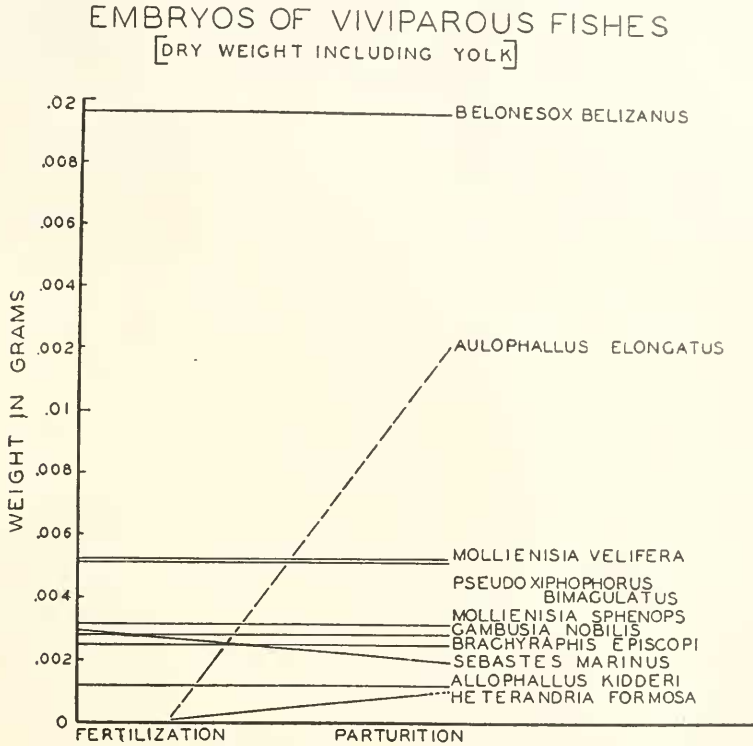


FIGURE 4. Additional species are shown in a manner identical to that described for Figure 3. The scale is enlarged to include species with greater embryonic weights. *Aulophallus* and *Heterandria* represent truly viviparous poeciliids. *Sebastes marinus* is a viviparous perch, a much larger fish than any of the others discussed and not a member of the Poeciliidae. It is included as an example of a truly ovoviparous fish.

be made for preserved material. Some embryos of *Gambusia affinis holbrookii* preserved since 1878 have changed very little in dry weight when compared with living ones studied in the laboratory. On the other hand, certain material collected in 1919 from the same place as the laboratory stock collected in 1941 has lost at least 70 per cent of its original dry weight. These specimens, however, were fixed in alcohol and preserved in strong alcohol (as high as 90 per cent). The 1878 material was fixed in formalin before it was transferred to the strong alcohol used as a preservative for fish by the Museum of Comparative Zoology. The material obtained from the Michigan Museum of Zoology was uniformly treated, having been fixed in 10 per cent formalin and changed to 70 per cent alcohol for storage.

The dry weight of the embryo in fishes treated in this manner closely approximates that of the living form. The data plotted for *Brachyrhaphis cascajalensis* in Figure 2 illustrate this.

In Figure 4, the *Poecilistes* species has been identified by Hubbs as being a race of *Poecilistes pleurospilus* from Guatemala. The *Poecilistes pleurospilus* listed is laboratory stock obtained from the Everglades Aquatic Nurseries, Tampa, Florida. The marked difference in average weight of these two groups of specimens, assuming them to be the same species, is probably due to actual racial differences rather than to fixation. A similar situation was found for *Mollicnisia*

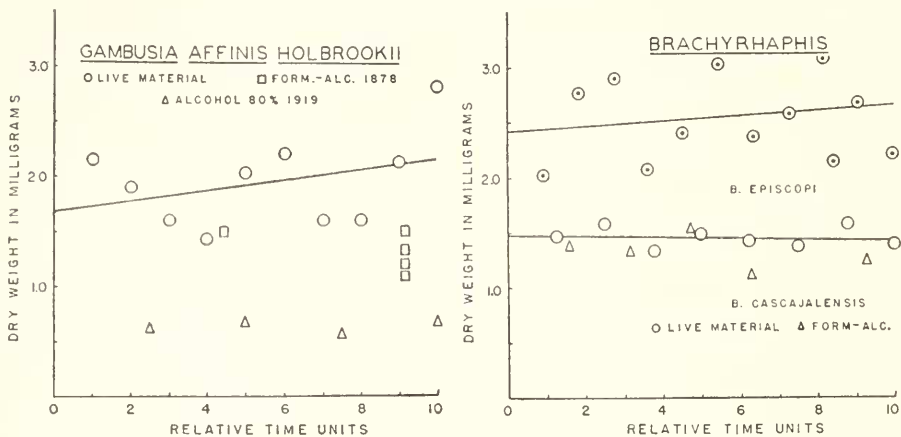


FIGURE 5. The dry weights of the embryo are plotted in a manner similar to Figure 1. The line is calculated from data represented by the circles. These represent embryos dissected out alive and dried immediately. The squares represent material preserved in formalin in 1878 and later transferred to the strong alcohol used in the Museum of Comparative Zoology, Cambridge, Mass. This varied between 75 and 90 per cent and was the sole preservative for specimens represented by the triangles.

FIGURE 6. The dry weights of the embryo are again plotted as in Figure 1. The triangles represent material preserved originally in 10 per cent formalin and later kept in 70 per cent alcohol. When weighed several months later there was excellent agreement with the values for living material studied at the time these were preserved. Most of the preserved material described has been handled in this manner.

*sphenops*. Two forms which could be distinguished by size, coloration, and mean weight of the embryos were taken from Gatun Lake. The mean weight of the larger form is plotted in Figure 3 as 3.05 mg. The smaller forms showed a very constant embryo weight averaging 1.58 mg. Despite these differences, Hubbs (1942) was able to find no usual taxonomic character or measurement to justify considering the smaller form a separate species.

Since the sperm in these fishes are viable in the ovary for several months, the time required to reach any given stage of development cannot be determined even in laboratory specimens. Therefore, in this study the embryonic weights were ranked in order of stage of development (cf. Scrimshaw, 1944). Since the data show that there is no consistent dry weight change during development, no error was introduced in the tabulation of the raw data. However, the assumption for

graphical and statistical purposes of an equal interval of time between the stages studied means the use of a relative rather than an actual time scale.

In the figures presented, the first stage was taken to represent the weight at fertilization and the last stage the weight at parturition. The time between was arbitrarily divided into ten units. Thus in the case of *Brachyrhaphis episcopi* where ten stages were studied, each stage in Figure 6 is separated by an arbitrary relative time unit of 1.0. In the case of *Allogambusia tridentiger* represented by fourteen stages (Fig. 2), the ten point scale must be divided into fourteen parts. Thus each stage is separated by 0.71 unit.

Using relative time units as the abscissa and dry weight in milligrams as the ordinate, the raw data were recorded graphically. It was apparent by inspection that the data for each species could be represented by a straight line. Accordingly these were fitted by the method of least squares and were of the form  $y = mt + k$ . The intercept ( $k$ ) of the straight line then represents the weight of the egg at fertilization. The slope ( $m$ ) describes the weight change during development.

TABLE I

Species	N	k	SE <sub>y</sub>	m	m°
1. <i>Gambusia affinis holbrooki</i>	10	1.676	.1127	.0502	2° 52'
2. <i>Darienichthyes dariensis</i>	6	1.925	.0584	-.0197	-1° 8'
3. <i>Poeciliopsis occidentalis</i>	12	1.923	.0456	.0027	0° 9'
4. <i>Poeciliopsis infans</i>	10	.937	.0108	.0046	0° 16'
5. <i>Aulophallus kidderi</i>	7	1.204	.0167	-.0040	-0° 13'
6. <i>Brachyrhaphis episcopi</i>	11	2.419	.1237	.0263	1° 30'
7. <i>Mollienisia sphenops</i>	12	3.045	.1495	.0204	1° 10'
8. <i>Dextripenis evides</i>	7	1.700	.0266	-.0350	-2° 0'
9. <i>Brachyrhaphis cascajalensis</i>	8	1.481	.0076	-.0055	-0° 19'
10. <i>Gambusia dovi</i>	10	1.667	.0479	-.0330	-1° 53'
11. <i>Allogambusia tridentiger</i>	14	1.005	.0132	-.0027	-0° 9'
12. <i>Xiphophorus helleri</i> *	21	1.841	.1602	-.0009	-0° 3'
Total	118			Average .0034	0° 12'

N, number of embryonic stages; K, intercept of development curve in milligrams; SE<sub>y</sub>, standard error or estimate of y in milligrams,  $SE_y = \sqrt{\frac{\sum d_y^2}{N-1}}$ ; m, slope of development line; m°, deviation of development line from horizontal in degrees.

\* Calculated from data of Bailey (1933).

As listed in Table I, the mean slope of all of these lines is only .0034. This is equivalent to an angle 0° 12', an almost imperceptible deviation from a slope of 0. In order that these small deviations may be more readily visualized, the actual angle in degrees equivalent to the calculated slope is also listed in Table I. From inspection of the data, it can be concluded that the random variations from a slope of 0 can be accounted for by biological variation and sampling error. No significant change in the dry weight of the embryo in the intact ovisac can be detected during development.

A further statistical basis for the above conclusions can be obtained by placing the data for the different species in numerically comparable form.<sup>5</sup> This consists of adjusting the data already calculated to an arbitrary standard. The weight at fertilization ( $k$ ) was made identical for each species and equal to unity ( $k_r$ ). All other statistics were scaled accordingly by multiplication with the ratio  $1/k$ . The resulting curves ( $y_r = m_r t + k_r$ ) are seen in Figure 7 and the new relative statistics are listed in Table II.

TABLE II

Species	$m_r$	$\hat{\sigma}m_r$	$t_{0.00}$	$t_{-.033}$	$P_{0.00}$	$P_{.033}$
1. <i>Gambusia affinis holbrookii</i>	.0300	.0213	1.409	2.961	.2	.02
2. <i>Darienichthyes dariensis</i>	-.0102	.0124	-.8270	1.841	.5	.15
3. <i>Poeciliopsis occidentalis</i>	.0014	.0068	.2032	5.028	.5	.001
4. <i>Poeciliopsis infans</i>	.0049	.0037	.7434	7.464	.5	.001
5. <i>Aulophallus kidderi</i>	-.0033	.0053	-.6324	5.653	.5	.001
6. <i>Brachyrhaphis episcopi</i>	.0109	.0154	.7049	2.845	.5	.02
7. <i>Mollienisia sphenops</i>	.0078	.0142	.5505	2.668	.5	.02
8. <i>Dextripenis evides</i>	-.0206	.0593	-.3473	.2093	.5	.5
9. <i>Brachyrhaphis cascajalensis</i>	-.0037	.0018	-2.044	6.088	.09	.001
10. <i>Gambusia dovii</i>	-.0198	.0091	-2.183	1.452	.05	.2
11. <i>Allogambusia tridentiger</i>	-.0027	.0035	-.7749	8.627	.5	.001
12. <i>Xiphophorus helleri</i> *	-.0005	.0190	-.0268	1.709	.5	.1

$k_r$ , common reference intercept,  $k_r = 1.000$  (omitted from table, but essential part of data identical for all species);  $m_r$ , slope of relative development curve,  $m_r = m \cdot 1/k$ ;  $\hat{\sigma}m_r$ , unbiased estimate of standard deviation of  $m_r$ ;  $t_{0.00}$ , Fisher- $t$  statistic,  $t_{0.00} = \frac{m_r - 0.00}{\hat{\sigma}m_r}$ ;  $t_{.033}$ , Fisher- $t$  statistic,  $t_{.033} = \frac{m_r - (-.033)}{\hat{\sigma}m_r}$ ;  $P_{0.00}$ , probability that the difference in slope between the observed curve and one of zero slope is due to random sampling error;  $P_{.033}$ , probability that the difference in slope between the observed curve and one of  $-.033$  slope (parturition weight = 2/3 fertilization weight) is due to random sampling error.

With the statistical measures for the 11 species adjusted in this manner, the impressions gained from the raw data can be evaluated. It was pointed out above that these fishes have been considered by most authors to be ovoviviparous. As will be discussed, this would mean that one-third of the nourishment originally available in the egg would be used before parturition for maintenance purposes. If this is so, the slope of a straight line development curve would be  $-0.033$  and the final dry weight would thus appear as  $\frac{2}{3}$  of the initial weight.<sup>6</sup>

The probability ( $P_{-.033}$ ) that the development curve of one of these species represents a chance deviation from a slope of  $-0.033$  can be determined by using the relation  $t_0 = [m_r - (0.033)/m_r]$  and tables of the Fisher- $t$  distribution. Examination of the  $P_{-.033}$  column in Table II shows that for most of the species examined the  $P$  is .05 or below. Thus, it is not probable that any of these curves represents a chance variation from a characteristic

<sup>5</sup> The statistical data in Table II have been calculated by Mr. James Rafferty of the School of Medicine and Dentistry, University of Rochester. The statistical treatment of the data has been carried out entirely by him and the text description of the methods employed has been prepared under his direction.

<sup>6</sup> The slope of such a line would be  $-0.33$  if the units were equal for ordinate and abscissa. Since there are ten relative time units to a common reference weight of unity, the figure for the calculated slope becomes  $-0.033$ .



slope of  $-0.033$ . To conclude that this group of curves as a whole shows chance deviation from such a slope is impossible. It should be apparent that the lines in Figure 7 do not represent a group of ovoviviparous species.

However, is the apparent grouping of the development curves about a slope of 0.00 significant? The probability ( $P_{0.00}$ ) that these lines represent chance variations from a characteristic slope of 0.00 can be found in a similar manner. It will be seen from Table II that the probability in most instances is .5 or better that this is the case. Taken as a whole, it is probable that the modal development curve for this group of species is a horizontal line. Thus the conclusion that there is no change in the weight of the embryo in the intact ovisac is substantiated.

The data for certain of the species require further comment. The data for species 2, 10, and 12 do show  $P_{-0.033}$  values of .1 - .2. Of these, only species 10, *Gambusia dovii*, shows a real deviation from a line of 0 slope (Fig. 7). In the case of species 8, *Dextripenis evides*, with a  $P_{-0.033}$  value of .5, there is enough initial variation in the weights that a deviation from a slope of 0.00 or  $-0.033$  is equally probable.

It might also appear from Figure 7 that species 1, *Gambusia affinis holbrookii*, has pro-

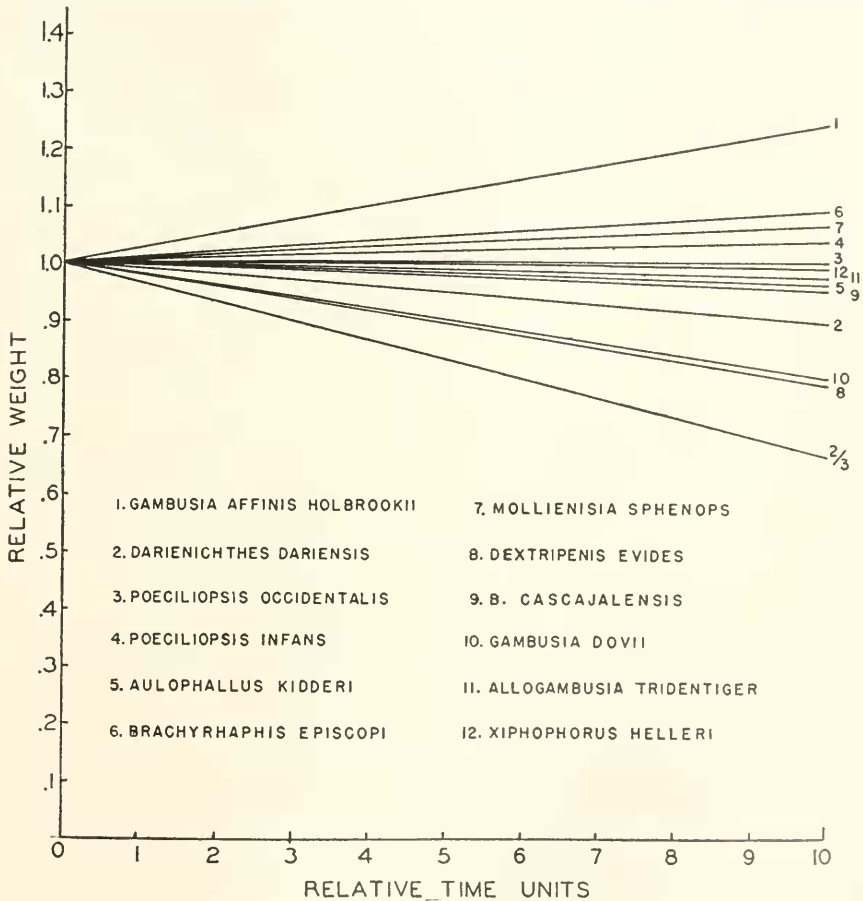


FIGURE 7. The weight data for the different species have been adjusted to an arbitrary standard so that the findings can be compared directly. The weight at fertilization ( $k$ ) was made identical for each species and equal to unity ( $k_r$ ). All other statistics were scaled accordingly by multiplication with the ratio  $1/k$ . Thus this new relative weight could be plotted as a function of relative time as used in the previous figures. The  $\frac{2}{3}$  line represents the line which the development curve of these species would follow if they were truly ovoviviparous.

gressed further than the other species toward true viviparity. While this possibility cannot be excluded, the  $P_o$  value of .20 may be taken to indicate that this deviation from zero slope may be a chance occurrence.

In several species it was not possible to secure enough stages to establish the mean dry weight of the embryo or the change in dry weight with development. The limited data for these species are summarized in Table III. The three females of *Lebistes* studied represented pure inbred laboratory stock and the average embryo weight of all three widely separated stages proved to be 2.3 milligrams. It is probable that most or all of the species in this table would fit into the group represented in Table I if complete data were available.

TABLE III  
Dry weight data for additional poeciliid species

Species	Number studied	Average weight (mg.)	$\sigma$	Adequacy of data
<i>Belonesox belizanus</i>	6	19.7	—	Range of 6.2–15.2 mg. for larva at parturition (6.2 value omitted from average)
<i>Brachyrhaphis terrabensis</i>	5	2.18	.81	Very narrow stage range and wide variation
<i>Gambusia nobilis nobilis</i>	8	2.8	.80	Only very early stages studied
<i>Lebistes reticulatus</i>	3		.0	Too few stages
<i>Mollienisia vivipara</i>	3	5.4	—	Few stages, very wide variation (3.4–6.3 mg.)
<i>Poecilia vivipara</i>	4	.96	.67	Too few stages
<i>Pseudoxiphophorus bimaculatus taeniatus</i>	5	5.3	—	Early stages only (Excessively low late stage value of 3.2 mg. omitted from average)

#### DISCUSSION

The variation in the weight of the intact ovisac at various embryonic stages seems to be a characteristic of the particular population of a species sampled. As described above, the standard error of  $y$  ( $SE_y$ ) is actually a composite of errors. Nevertheless, it is primarily a reflection of the physiological and genetic variability to be found within the representatives of the species studied. It is probably valid to compare the inherent genetic variability of one species sample with that of another by means of this statistic. When the  $SE_y$  is small enough to minimize overlapping, the weight of the embryos should occasionally prove of taxonomic use.

The experimental data show the weight of the embryo at parturition to be the same as that of the egg at fertilization. Thus, quantitatively at least, the full amount of food material in the egg is converted into the substance of the embryo at the time of hatching. However, it is known that the food requirement of the embryo for maintenance metabolism during development is considerable.

Gray (1928) obtained for the plastic efficiency coefficient (P.E.C.) of the trout a figure of 0.63. This he believes to be a universal value for the synthesis of

protoplasm. This coefficient is based on the dry weight of the yolk that can be accounted for by the dry weight of the fully developed larva. It represents the relative cost in weight units of building the tissues of the embryo. The higher the coefficient, the smaller the amount of burned substance in relation to the amount of nourishment initially available. Table IV summarizes the values for P.E.C. which have been reported. These values suggest that the mother must also contribute an amount of nourishment equal to about one-third the initial weight in the poeciliid fishes studied.

TABLE IV  
*Plastic efficiency coefficients of development*

Species	Author	Coefficient
Trout ( <i>Salmo fario</i> )	Gray (1926)	.63 embryo
Chick ( <i>Gallus domesticus</i> )	Murray (1926)	.68 embryo
Frog ( <i>Rana temporaria</i> )	Fauré-Fremiet & Dragoiu (1923)	.58 embryo
Silkworm ( <i>Bombyx mori</i> )	Farkas (1903)	.59 embryo
Mould ( <i>Aspergillus niger</i> )	Terroine & Wurmser (1922)	.59 normal growth
Viviparous perch ( <i>Sebastes marinus</i> )	Hsiao (unpublished data)	.66 embryo
Trout ( <i>Savelinus fontinalis</i> )	Gortner (1912)	.74 embryo (heat dried)

The maintenance cost of development is also illustrated by the various measures of the energy required during development. This subject has been well summarized by Needham (1931). Table V shows the values which have been obtained experimentally for one type of energy coefficient, the apparent energetic

TABLE V  
*Apparent energetic efficiency of embryonic development*

Species	Author	Efficiency
Chick ( <i>Gallus domesticus</i> )	Tangl (1903)	62.9%
Chick ( <i>Gallus domesticus</i> )	Murray (1926)	67.0
Fundulus ( <i>Fundulus heterolitus</i> )	Glaser (1912)	52.8*
Frog to hatching ( <i>Rana temporaria</i> )	Fauré-Fremiet & V. du Stree (1921)	82.0
Frog to end of yolk ( <i>Rana temporaria</i> )	Fauré-Fremiet & Dragoiu (1923)	51.0

\* This value was obtained by assuming that the same energy relationships prevailed for the use of the final half of the yolk as for the initial half. This figure is certainly too low.

efficiency (A.E.E.), which gives the relation between the chemical energy in the fertilized egg and that combusted during development. For example, the figure cited by Tangl for the chick would indicate simply that 37.1 per cent of the original energy stored in the egg was lost by the time the yolk was consumed and the embryo well formed. All of the available evidence indicates that it is legitimate to generalize and to conclude that in the course of embryonic growth of animals developing from a yolked egg, about one-third of the energy initially available is expended for maintenance metabolism.

The evidence that this relationship is also true for viviparous animals is more difficult to obtain. Rubner (1908) derived values, in part theoretical, for the effi-

ciency of seven common mammals (horse, cow, sheep, pig, dog, cat and rabbit). The agreement of these values with the above data is sufficiently close to permit Needham (1931) to conclude that the real energetic efficiency (R.E.E.) of all embryos, mammalian as well as non-mammalian, is about .66. The R.E.E. involves a correction for basal metabolism, but for poikilothermic animals (and embryos such as those of the chick which are essentially cold blooded for the greater part of their prenatal life) it is so close to the A.E.E. that the two can be considered together.

An energy loss of about 34 per cent in the poeciliid fishes studied should be reflected in an appreciable decrease in the total amount of organic matter. The fact that no change in dry weight can be detected points to an exact correspondence between the weight of food used for maintenance and that of the nourishment supplied by the mother. But why should the majority of species in the family Poeciliidae stop at this identical point in the development of viviparity? Some similar factor or factors must be limiting the growth of the embryo to the initial weight of the egg in all of these species.

This might be the case if some essential factor in the yolk were present in a limiting quantity. If this factor could not be supplied by the mother, no amount of energy from her would permit the embryo to grow larger than the initial supply of this limiting substance would allow. Such might be the case if, for example, only simple sugars and certain amino acids were able to cross the placental barrier. Then certain of the essential amino acids, fats, vitamins or other factors might be available to the embryo in restricted quantities. However, the exact correspondence between initial and final weight in nearly all the species is difficult to understand on this basis.

In these fish the nourishment must pass through five to seven tissue layers: the maternal endothelium, maternal connective tissue (theca), maternal epithelium (follicular epithelium), possibly the chorion and vitelline membrane, yolk sac epithelium, embryonic endothelium, and probably some embryonic connective tissue. This situation resembles most closely the epitheliochorial type of placenta found in such animals as the pig. Despite the number of membranes in this type of placenta all of the materials necessary for growth and maintenance pass from the mother to the fetus across this barrier.

In some mammals, the materials most important for the elaboration of embryonic tissues, the amino acids, pass through the placenta against a concentration gradient in a manner suggesting a special mechanism (Needham, 1942). If a completely viviparous fish such as *Heterandria formosa* possessed such a mechanism, the steady gain in weight of its embryos can be understood. If, on the other hand, the fish discussed here lack such a mechanism, the failure of their embryos to gain weight might also be accounted for.

It is also of interest to note that carbohydrate is absent from eggs of oviparous fishes. Needham (1931), in summarizing the available data, expresses the belief that this is true because of the impossibility of supplying an oviparous form with sufficient sugar for embryonic metabolism without vastly increasing the size of the egg. However, there is no reason to believe that carbohydrate would not be used by their embryos in preference to the stores of fat and protein, if it were available. In the case of the mammalian fetus, Windle (1940, page 212) concludes from the available evidence that the fetus of the mammal practices a rigid glycogen

economy at the expense of its mother's dextrose, drawing upon its own liver only in emergencies. It seems reasonable to expect that carbohydrate would also be the most readily available food which the embryo of the viviparous fish can obtain from the mother. Perhaps in the species discussed, carbohydrate from the mother exerts some sparing action on the tissue building materials of the yolk.

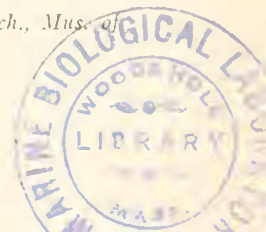
The discussion has been carried to this point to suggest something of the direct study which should be made on the functioning of the pseudo-placental barrier in these forms. The controlling mechanisms which are actually operating in the development of the species reported will not be clear until such experiments are carried out.

#### SUMMARY

In poeciliid fishes the embryos are retained within the ovarian follicles until parturition. When the dry weight of the intact follicle is determined at various stages of development, no significant weight change can be demonstrated in the 18 species studied. This is in marked contrast to the situation in a truly ovoviviparous fish where one-third of the initial weight of the egg is used for maintenance during development. Accordingly, it is concluded that all of the species studied receive nourishment from the mother and hence are not ovoviviparous in the strict sense of the word. Furthermore, they apparently receive only as much nutriment, quantitatively at least, as they require for their maintenance metabolism. Some limiting mechanism must be operating, but its nature is not known. This situation is contrasted with that in *Heterandria formosa* and *Aulophallus elongatus*, members of the family which have developed nearly complete dependence on the mother for nourishment during development.

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